

AMENDMENTSIn the Claims:

Claim 1. (Currently Amended) Isolated photoprotein containing an amino acid sequence which:

- a) is able to bind coelenterazine and calcium, producing bioluminescence;
- b) is identical by at least 90% to SEQ ID NO: 1 (Clytin);
- c) is in sequence alignment with SEQ ID NO: 1 (Clytin), presents and comprises one of the following single or multiple substitutions (the residue positions are referred with reference to SEQ ID NO: 1):
 - i) C₅₄→S;
 - ii) S₁₃₂→C;
 - iii) K₄₈→R, N₁₉₅→D;
 - iv) Q₆₈→R, A₉₀→V, T₁₈₄→I;
 - v) Y₈₂→F, K₁₁₀→N, F₁₂₅→L, S₁₄₉→R;
 - vi) G₁₄₂→C;
 - vii) I₅₃→V, S₁₄₉→R;
 - viii) N₁₈→D, I₄₀→V, K₅₆→R;
 - ix) Gly₅₈→Glu, Asp₆₉→Val, Ala₇₀→Cys, Lys₇₆→Arg, Lys₇₇→Gly, Ile₇₈→Cys, Asp₈₁→Glu, Val₈₆→Ile, Glu₈₇→Ala, Ala₉₀→Gln, Val₉₂→Leu, and Glu₉₇→Gln or a functional derivative or fragment thereof.

Claim 2. (Original) The photoprotein of claim 1, containing an amino acid sequence identical by at least 95% to SEQ ID NO: 1.

Claim 3. (Original) The photoprotein of claim 2, containing an amino acid sequence identical by at least 98% to SEQ ID NO: 1.

Claim 4. (Original) The photoprotein of claim 3, containing an amino acid sequence which is selected from the group consisting of SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10.

Claim 5. (Previously Presented) A photoprotein according to claim 1, wherein said amino acid sequence is fused to a mitochondrial target sequence.

Claim 6. (Withdrawn) An isolated polynucleotide encoding a photoprotein according to claim 1.

Claim 7. (Withdrawn) The polynucleotide of claim 6, having the sequence of SEQ ID NO: 11, 12, 13, 14, 15, 16, 17, 18, 19.

Claim 8. (Withdrawn) An expression vector containing a polynucleotide according to claim 6.

Claim 9. (Withdrawn) A prokaryotic or eukaryotic host cell containing the vector of claim 8.

Claim 10. (Withdrawn) A mammalian host cell according to claim 9.

Claim 11. (Withdrawn) A method in vitro for detecting changes in intracellular calcium concentration which comprises:

- a) providing a cell expressing a photoprotein according to claim 1;
- b) contacting the cell with an agent stimulating calcium influx or calcium release from intracellular stores;
- c) detecting the photoprotein bioluminescence.

Claim 12. (Withdrawn) A method of screening compounds modulating intracellular calcium concentration, which comprises:

- a) providing a cell expressing a photoprotein of claim 1;
- b) contacting the cell with the candidate compound;
- c) detecting the bioluminescence of the photoprotein.

Claim 13. (Withdrawn) A method according to claim 11, which is carried out in a high-throughput format.

Claim 14. (Withdrawn) A method according to claim 13, which is carried out with a high throughput optical screening apparatus suited for multi-sample analysis.

Claim 15. (Withdrawn) The use of a photoprotein according to claim 1 as intracellular calcium indicator.

Claim 16. (Withdrawn) The use of a photoprotein according to claim 15 in a cell-based high throughput assay.

Claim 17. (Withdrawn) The use of a photoprotein according to claim 1 for the preparation of a diagnostic composition.